

Amendment to the Specification

► Please amend [0003] as follows:

-- (Amended) In 1998, it was firstly reported that fluorescence intensity of GFP was maintained even after inserting foreign protein or a part of protein or peptides thereto at one or more sites (see NAR 26:623-630). Since then, molecules belonging to the group of the inserted fluorescence proteins, which were developed by R Tsien et al., have been used. YFPins and Camgaroo, which was made by inserting calmodulin to the YFPins, were representative inserted fluorescence proteins designed by R Tsien et al. ~~As for the inserted green fluoresce proteins reported by R Tsien et al., the 145th amino acid sequence of Tyrosine was replaced by "GGTGEL" where restriction enzymes (such as, KpnI and Sad) recognition sites were introduced in order to clone foreign nucleic acid sequences (see PNAS 96:11241-11246).~~ The mutant fluorescence proteins, however, did not show fluorescence activities at 37° c., while they displayed fluorescence activities at 28° C. Thus, they could not be used as biosensors in mammalian cell to measure activities of any desired materials. R Tsien et al. also reported that Camgaroo 2 (Q69M mutant), which was made by substituting 69th amino acid sequence of Glutamine with Methionin, represented fluorescence even at 37° C. However, the fluorescence intensity of the Camgaroo 2 was so weak that it could not be used in the measurement of calcium at a single cell level. Accordingly, there has been the need to find a novel inserted fluorescence protein having even stronger fluorescence. --

► Please amend [0005] as follows:

-- (Amended) It is known that caspase recognizes and cleavages a protein at the site of amino acids following aspartic acid. ~~Caspase-1,4,5,13 cleavage WEHD site, caspase-2,3,7 cleavage DEXD, and caspase-6,8,9,10 cleavage I(IV/L)EXD.~~ In 1998, Xu et al. detected caspase-3 (CPP32) activity using FRET (Fluorescence Resonance Energy Transfer) that was caused by placing DEVD (SEQ ID No.: 17) amino acid sequence between GFP and BFP (see NAR 26:2034-2035). Also, BD bioscience clontech designed a system to monitor the activity of caspase-3 through tracing and investigating the YEP within a cell by fusing DEVD-YEP and nuclear export sequence (BD bioscience clontech, PR1Z499W). However, in detecting caspase activity using FRET, signal/noise (S/N) ratio was too low for practical application in the assay system. In addition, this assay system, which was basically based on protein movement in a cell,

required relatively expensive device, and it was difficult to digitize the enzyme activity since the detected results were secondary signals. Accordingly, there have been great needs to find more efficient and cost effective cell-based assay system to detect and analyze the activities of materials.--

► Please amend [0014] as follows:

-- (Amended) Thus, the obtained and selected mutant inserted yellow fluorescence protein, according to the above method, is characterized by including "YGGSGAS" (partial sequence of SEQ ID No.:1) at 145th amino acid site, wherein the amino acid site is insertion region of foreign protein or a part of protein. This mutant inserted fluorescence protein is named as Y-Citrine (SEQ.ID No.: 1). The insertion region is designed to function as a binding site that has little electricity in comparison to that of the conventional inserted fluorescence protein. Since the insertion region has restriction enzyme recognition sites, which cannot be found in conventional vectors, it is possible to clone numerous genes into the vector with just one cloning process. --

► Please amend [0021] as follows:

-- (Amended) Furthermore, a caspase sensor is provided using Peridot to monitor the activity of caspase in a cell. For this purpose, DEVD (SEQ ID No.: 17) amino acid sequence is inserted into the Peridot, and it is named DEVDins. The produced DEVDins is transferred to CHO-K1 (Chinese hamster ovarian) cell line, and then DEVDins expressing cell line is selected and named as CHO-K1-DEVDins. After that, the selected cell line is treated with cell death inducing agent, and the activity of caspase-2/3/7 is detected using quantitative fluorescence image analysis (see FIG. 3). Thus, recombinant fluorescence proteins including caspase recognition amino acid sequences, ~~for example, WEHD for caspase 1/4/5/13, DEXD for caspase 2/3/7 and I(!VIL)EXD for caspase 6/8/9/10,~~ are provided.--

► Please amend [0036] as follows:

-- (Amended) A caspase sensor, which can be used in monitoring the activity of caspase, was prepared in this example using inserted fluorescence protein. DEVD amino acid sequences (SEQ ID No.: 17), which were recognized by caspase 2/3/7, were introduced into insertion region of the inserted fluorescence protein so that the fluorescence intensity of prepared biosensor can be in direct proportion to the change of the activities of caspase. With the prepared biosensor, it was

possible to detect activity of caspase 2/3/7 under fluorescent microscope and was possible to digitize the activities. As mentioned in the example 1, a pair of primers of BamHI/DEVD F 5'-GGGGGATCCGCCATCAAGAATGAAGGAAAG AGAAAAGGCGACGAGGTG -3' (SEQ. ID No.: 14) and NheI/DEVD R 5'-GGGGCTAGCG GCCACTTCAT CTGTTCCATC CACCTCGTCG CCTTTTCTC-3' (SEQ. ID No.: 15) were synthesized and then were combined. Next, the combined primers were cloned into the insertion region in pER1 with restriction enzyme, and the resultant product was named DEVDins (SEQ. ID No.: 16). After transferring the DEVDins to CHO-K1 (Chinese hamster ovarian, ATCC #CCL61) cell line, the cell line was treated with cell death inducing agent in order to activate caspase to monitor the fluorescence intensity. As a result, it was observed that the fluorescence intensity of the prepared biosensor decreased due to the denaturation of the inserted fluorescence protein by the activated caspase.--

► Please replace the sequence listing starting after [0041] with the following replacement sequence listing:

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<110> Neurogenex Co., Ltd.
<120> ENHANCED INSERTED YELLOW FLUORESCENCE PROTEIN AND ITS
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<140> US 10/506,925
<141> 2004-09-07
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<213> Artificial Sequence

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<223> y-citrine of fluorescence protein

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20 25 30

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35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr

50 55 60

Phe Gly Tyr Gly Leu Met Cys Phe Ala Arg Tyr Pro Asp His Met Lys

65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu

85 90 95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu

100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly

115 120 125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

130 135 140

Asn Tyr Gly Gly Ser Gly Ala Ser Asn Ser His Asn Val Tyr Ile Met

145 150 155 160

Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His

165 170 175

Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn

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Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu

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 Phe Gly Tyr Gly Leu Met Cys Phe Ala Arg Tyr Pro Asp His Met Lys
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